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The nonextractive immunoassay techniques developed with enzyme immunoassay procedures were extended to include fluorescent immunoassay of sorbed contaminants. The variety of substrates to which the contaminant was sorbed was increased to include: St. Peters sandstone, montmorillonite, and black shale. Autofluorescence of the substrates was addressed, and 2,4-dinitrobenzene was found not to migrate into the montmorillonite crystal layers. Additional sample preparation was needed for the clay samples: significantly more blocking solution was needed to reduce background nonspecific binding of the immunochemicals. Two contaminants were used throughout the grant period -- 2,4-dinitrobenzene, and pyrene.

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AUGMENTATION AWARD FOR MONOCLONAL ANTIBODY DETECTION OF CHLORINATED BENZENES ON CONTAMINATED SEDIMENTS

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31 October 1996

AASERT Final Technical Report

Prepared for
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
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Progress of AASERT Students

The AASERT students have been using a Zeiss Axiophot epifluorescent microscope to examine the feasibility of fluorescent immunoassay (FIA) techniques for visualization of contaminants sorbed to soil particles. The supported students also employed enzyme immunoassay techniques to supplement the fluorescent immunoassay efforts.

The first student supported by the AASERT grant graduated with a Master of Science in Civil Engineering in December 1994. His M.S. thesis is titled "Fluorescent Immunoassay Visualization of Sorbed Pollutants" (author = Wesley K. Moore, 45 pp.). The conclusions of his research were: 1. R-phycoerythrin (RPE) was the best fluorochrome for the immunoassay application; 2. contamination was observed to occur in patches, rather than a continuous distribution; 3. nonspecific fluorescence and autofluorescence problems were unresolved in his research on natural materials, such as clay and sand.

The contaminant sorbed on the solids was 2,4-dinitrobenzene sulfonate (DNB), and the primary antibody was anti-DNB developed in rabbit (rb $\bar{a}$DNB) (Sigma). The samples were contaminated by soaking the soil samples in a pH 10 DNB solution. The labeled secondary antibody was commercially-obtained antibodies to rabbit developed in goat labeled with R-phycoerythrin (gt $\bar{a}$rb-RPE). All results are photo documented with daylight ASA400 Ektachrome slide film.

Clean and contaminated samples are first blocked using a protein solution to prevent nonspecific attachment of antibodies during the procedure. The samples are then incubated and agitated in the primary antibody solution (rb $\bar{a}$DNB). The samples are then washed to remove unbound antibodies, and the fluorochrome-labeled secondary antibody (gt $\bar{a}$rb-RPE) is added, and the sample and solution are incubated and agitated. The sample is washed again, and can then be viewed under the epifluorescence microscope.

An oral presentation titled "Fluorescent Immunoassay Visualization of Sorbed Pollutants" was given by W. K. Moore (AASERT student) at the Ninth Annual Hazardous Waste Research Conference, 8-10 June 1994, Montana State University, Bozeman, Montana.

Two graduate students were put on the AASERT grant in March 1995. Both students are working to resolve the experimental problems identified by the first AASERT student. One student focussed on clays. He examined montmorillonite contaminated with 2,4-dinitrobenzene sulfonate by X-ray diffraction to determine if the contaminant migrated into the interstitial clay layers where it would be unavailable for detection by immunoassay. He found that the dinitrobenzene does not appear to migrate into the clay.

An unexpected result of this line of research was the sample preparation need for clay samples. Three sequential blocking steps were needed to minimize nonspecific binding. When the original (parent grant) research began, we found that a 10x increase in skim milk content in the blocking solution was needed to prepare brick and sand samples. When blocking polystyrene microtiter plates, a 3 g/l solution of dry skim milk is sufficient to block nonspecific binding. For sand and brick chips, 30 g/l was needed. For clay, three 30 g/l block solutions were needed. His masters thesis is titled, "Problems Created by the Mineralogy of Clay on a Nonextractive Enzyme Linked Immunosorbent Assay" (author = Patrick E.G. Morgan, May 1996, 68 pp.).

An oral presentation titled, "Immunochemical Techniques to Detect Contaminants on Clays," was presented by Patrick Morgan (AASERT student) at the North-Central Section of the

Geological Society of America 30th Annual Conference, held in Ames, Iowa during 2-3 May 1996.

The another AASERT student is applying the fluorescent immunoassay visualization to black shale. His masters thesis is titled, "Nonextractive Immunoassay Detection and Visualization of Sorbed Pyrene on a Solid Medium" (author = William T. Trefz, expected graduation May 1997). Black shale was chosen as an ideal dark-background substrate for the epifluorescent microscopy. Commercially-produced antibodies to pyrene (developed in rabbit) were purchased from Serotec. Pyrene was chosen because it is hydrophobic, a toxic pollutant, and naturally fluorescent. The natural fluorescence of pyrene was used to visually verify the contamination of the samples. The student is using ELISA (enzyme-linked immunoassay) as well as fluorescent immunoassay to assess the viability of using immunoassays to detect sorbed pyrene. Preliminary results indicate that sorbed pyrene can be detected using nonextractive immunoassay techniques.

Two other graduate students worked briefly on this project in support capacities during the summer of 1996: Rodney Jensen and David Roberts. Both are U.S. citizens.

Academic Performance of AASERT Student

The students supported under the AASERT grant have all maintained good grade point averages during the grant period.

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